INTERFERING RNA MOLECULES

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 14/977,710, filed Dec. 22, 2015, which is a continuation of U.S. patent application Ser. No. 14/578,636, filed on Dec. 22, 2014, now U.S. Pat. No. 9,222,092, issued Dec. 29, 2015, which is a continuation of U.S. patent application Ser. No. 13/692,178, filed on Dec. 3, 2012, now U.S. Pat. No. 8,933,215, issued Jan. 13, 2015, which is a continuation of U.S. patent application Ser. No. 12/986,389, filed on Jan. 7, 2011, now U.S. Pat. No. 8,324,370, issued Dec. 4, 2012, which is a continuation of U.S. patent application Ser. No. 12/200,296, filed on Aug. 28, 2008, now U.S. Pat. No. 7,893,245, issued Feb. 22, 2011, which is a continuation of U.S. patent application Ser. No. 10/633,630, filed on Aug. 5, 2003, now U.S. Pat. No. 7,452,987, issued Nov. 18, 2008, which claims the benefit of U.S. Provisional Application No. 60/402,541, filed Aug. 12, 2002, which claims priority to European application No. 02017601.2, filed Aug. 5, 2002, and European application No. 03008383.6, filed Apr. 10, 2003. Each of these applications are incorporated herein by reference in their entirety, including all figures, tables and amino acid or nucleic acid sequences.

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name: 680132000107seqlist.txt, date recorded: May 1, 2017, size: 85 KB).

FIELD OF THE INVENTION

The invention provides novel forms of interfering ribonucleic acid molecules having a double-stranded structure. The first strand comprises a first stretch of contiguous nucleotides that is at least partially complementary to a target nucleic acid, and the second strand comprises a second stretch of contiguous nucleotides that is at least partially identical to a target nucleic acid. Methods for using these molecules, for example for inhibiting expression of a target gene, and pharmaceutical compositions, cells and organisms containing these molecules also are provided.

BACKGROUND OF THE INVENTION

RNA-mediated interference (RNAi) is a post-transcriptional gene silencing mechanism initiated by double stranded RNA (dsRNA) homologous in sequence to the silenced gene (Fire (1999), Trends Genet 15, 358-63, Tuschl, et al. (1999), Genes Dev 13, 3191-7, Waterhouse, et al. (2001), Nature 411, 834-42, Elbashir, et al. (2001), Nature 411, 494-8, for review see Sharp (2001) Genes Dev 15, 485-90, Barstead (2001), Curr Opin Chem Biol 5, 63-6). RNAi has been used extensively to determine gene function in a number of organisms, including plants (Baulcombe (1999) Curr Opin Plant Biol 2, 109-13), nematodes (Montgomery, et al. (1998), Proc Natl Acad Sci U.S.A. 95, 15502-7), *Drosophila* (Kennerdell, et al. (1998), Cell 95, 1017-26, Kennerdell, et al. (2000). Nat Biotechnol 18, 896-8). In the nematode *C. elegans* about one third of the genome has already been subjected to functional analysis by RNAi (Kim (2001), Curr Biol 11, R85-7, Maeda, et al. (2001), Curr Biol 11, 171-6).

Until recently RNAi in mammalian cells was not generally applicable, with the exception of early mouse development (Wianny, et al. (2000), Nat Cell Biol 2, 70-5). The discovery that transfection of duplexes of 21-nt into mammalian cells interfered with gene expression and did not induce a sequence independent interferon-driven anti-viral response usually obtained with long dsRNA led to new potential application in differentiated mammalian cells (Elbashir et al, (2001). Nature 411, 494-8). Interestingly these small interfering RNAs (siRNAs) resemble the processing products from long dsRNAs suggesting a potential bypassing mechanism in differentiated mammalian cells. The Dicer complex, a member of the RNAse III family, necessary for the initial dsRNA processing has been identified (Bernstein, et al. (2001), Nature 409, 363-6. Billy, et al. (2001), Proc Natl Acad Sci U.S.A. 98, 14428-33). One of the problems previously encountered when using unmodified ribooligonucleotides was the rapid degradation in cells or even in the serum-containing medium (Wickstrom (1986), J Biochem Biophys Methods 13, 97-102, Cazenave, et al. (1987), Nucleic Acids Res 15, 10507-21). It will depend on the particular gene function and assay systems used whether the respective knock down induced by transfected siRNA will be maintained long enough to achieve a phenotypic change.

It is apparent, therefore, that synthetic interfering RNA molecules that are both stable and active in a biochemical environment such as a living cell are greatly to be desired.

SUMMARY OF THE INVENTION

It is therefore an object of the present invention to provide compositions and methods using interfering RNA molecules having enhanced stability.

In accomplishing this object, there has been provided, in accordance with a first aspect of the present invention, a ribonucleic acid comprising a double stranded structure whereby the double-stranded structure comprises a first strand and a second strand, whereby the first strand comprises a first stretch of contiguous nucleotides and whereby said first stretch is at least partially complementary to a target nucleic acid, and the second strand comprises a second stretch of contiguous nucleotides whereby said second stretch is at least partially identical to a target nucleic acid, and whereby the double stranded structure is blunt ended.

In accordance with a second aspect of the present invention there has been provided a ribonucleic acid comprising a double stranded structure whereby the double-stranded structure comprises a first strand and a second strand, whereby the first strand comprises a first stretch of contiguous nucleotides and whereby said first stretch is at least partially complementary to a target nucleic acid, and the second strand comprises a second stretch of contiguous nucleotides, whereby said second stretch is at least partially identical to a target nucleic acid whereby the first stretch and/or the second stretch have a length of 18 or 19 nucleotides.

In an embodiment of the ribonucleic acid according to the first aspect of the invention the first stretch and/or the second stretch have a length of 18 or 19 nucleotides.

In a further embodiment of the ribonucleic acid according to the first aspect of the invention the double stranded structure is blunt ended on both sides of the double strand.